

DNA of a given, pre-selected pair. In order to verify that the library populations encode the designed amino acids with the expected frequency, single clones from each library were randomly picked and sequenced before selection. No statistically significant biases were detected. Seventy to 80% of each library had no mutations or frame-shifts, and thus the library-vs-library combination yielded approximately 50% correct sequence combinations. Thus, the experimental library-vs-library size of correct pairs is estimated as 2×10^6 .

Replace the paragraph beginning "The peptides WinZip-A1" on page 27 of the specification with the following paragraph.

The peptides WinZip-A1: Ac-STTVAQLEEKVKTLRAQNYELKSRVQRLRE-QVAQLAS-NH₂ (SEQ ID NO. 35) and WinZip-B1: Ac-STSVDELQAEVDQLQDENYALKTKVAQLRKKVEKLSE-NH₂ (SEQ ID NO. 36) were synthesized (Applied Biosystem 431A) and purified by reversed-phase HPLC. Electrospray mass spectrometry confirmed purity and identity of the peptides with a mass deviation of less than 1 Da. Peptide concentrations were determined by tyrosine absorbance in 6 M GdnHCl (39).

REMARKS

Applicants have inserted new Sequence Listing pages 1-12 submitted herewith and have replaced the paragraphs on pages 3, 5, 6, 18 and 27 of the specification with replacement paragraphs indicating SEQ ID NOs. An Appendix of Amendments is attached herewith showing all the amendments. In the Appendix, additions are indicated by underlining. This amendment does not introduce new matter.

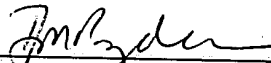
Applicants respectfully request entry of the above amendment and early allowance of the pending claims.

The Notification of Missing Requirements states that the current oath or declaration does not comply with 37 C.F.R. § 1.497(a) and (b) in that it is not executed in accordance with either 37 C.F.R. § 1.66 or 37 C.F.R. § 1.68. Accordingly, applicants enclose an executed Declaration and Power of Attorney and a check for \$130 to pay for a surcharge set forth in 37 C.F.R. § 1.492(e).

Additionally, applicants have complied with the requirements under 37 C.F.R. §§ 1.821-1.825, as indicated in the Notification of Missing Requirements. The Notification of Missing Requirements states that applicants must provide a Sequence Listing (on paper and a computer readable form), an amendment directing its entry of the Sequence Listing into the specification and a Statement that the content of the paper and computer readable copies is the same and includes no new matter. Accordingly, applicants enclose new Sequence Listing pages 1-12, a Computer Readable Form submission of same and the required Statement.

The Notification of Missing Requirements also states that an additional claim fee of \$298 as a non-small entity, including any required multiple dependent claim fee, is required. Accordingly, applicants have enclosed a check for \$298 to pay for the additional claim fees.

Respectfully submitted,



James F. Haley, Jr. (Reg. No. 27,794)

Attorney for Applicants

Stanley Liang (Reg. No. 43,753)

R. Minako Pazdera (Reg. No. 46,984)

Agents for Applicants

c/o FISH & NEAVE (Customer No. 1473)

1251 Avenue of the Americas

New York, New York 10020-1104

Tel.: (212) 596-9000

Fax.: (212) 596-9090

Appendix of Amendments

Thus, the present invention relates to a method for the identification of hetero-associating (poly)peptides comprising the steps of:

(a) providing a library A of (poly)peptides/proteins comprising (poly)peptides A_m having the general formula:

VAQLXEXVKTLXAXZYELXSXVQRLXEXVAQL (SEQ ID NO. 1)

wherein X represents a mixture of E, K, Q, and R, and wherein Z represents a mixture of N and V,

(b) providing a library B of (poly)peptides/proteins comprising (poly)peptides B_n having the general formula:

VDELXAXVDQLDXZYALXTXVAQLXKXVEKL (SEQ ID NO. 2)

wherein X represents a mixture of E, K, Q, and R, and wherein Z represents a mixture of N and V;

(c) combining in a common medium the (poly)peptides/proteins of said libraries A and B; and

(d) screening or selecting for a screenable or selectable property caused by the hetero-association of a (poly)peptide A_m with a (poly)peptide B_n .

In another embodiment, the present invention relates to a hetero-associating (poly)peptide A_m taken from the list of:

WINZIPA1: VAQLEEKVKTLRAQNYELKSRVQRLREQVAQL (SEQ ID NO. 3)

WINZIPA2: VAQLRERVVKTLRAQNYELESEVQRLREQVAQL (SEQ ID NO. 4)

WINZIPA3: VAQLQEKVKTLRARNYELKSEVQRLEEKVAQL (SEQ ID NO. 5)

WINZIPA4: VAQLEEQVKTLQARNYELKSKVQRLKEKVAQL (SEQ ID NO. 6)

WINZIPA5: VAQLEERVVKTLRAQNYELKSKVQRLREQVAQL (SEQ ID NO. 7)

WINZIPA6: VAQLEEQVKTLAENYELKSKVQRLRERVAQL (SEQ ID NO. 8)

WINZIPA7: VAQLQEQVKTLAQNYLESEVQRLKEQVAQL (SEQ ID NO. 9)

WINZIPA8: VAQLEERVKTLKAENYLESEVQRLKERVVAQL (SEQ ID NO. 10)

WINZIPA9: VAQLEEKVKTLKAKNYELKSKVQRLKEKVAQL (SEQ ID NO. 11)

WINZIPA10: VAQLQEEVKTLQAENYELRSEVQRLEEEVAQL (SEQ ID NO. 12)

WINZIPA11: VAQLRERVKTLRARNYELQSKVQRLKERVAQL (SEQ ID NO. 13)

Furthermore, the present invention relates to a hetero-associating (poly)peptide B_n taken from the list of:

WINZIPB1: VDELQAEVDQLQDENYALKTKVAQLRKKVEKL (SEQ ID NO. 14)

WINZIPB2: VDELKAEVDQLQDQNYALRTKVAQLRKEVEKL (SEQ ID NO. 15)

WINZIPB3: VDELEAEVDQLKDQNYALKTKVAQLQKQVEKL (SEQ ID NO. 16)

WINZIPB4: VDELRAKVDQLQDENYALETEVAQLQKRVEKL (SEQ ID NO. 17)

WINZIPB5: VDELEAEVDQLEDQNYALQTRVAQLEKRVEKL (SEQ ID NO. 18)

WINZIPB6: VDELKAKVDQLKDKNYALRTKVAQLRKKVEKL (SEQ ID NO. 19)

WINZIPB7: VDELRAQVDQLQDKNYALRTRVAQLKKRVEKL (SEQ ID NO. 20)

WINZIPB8: VDELQAEVDQLQDQNYALRTQVAQLKKKVEKL (SEQ ID NO. 21)

WINZIPB9: VDELRAQVDQLEDQNYALETQVAQLEKEVEKL (SEQ ID NO. 22)

WINZIPB10: VDELQAKVDQLKDENYALQTKVAQLQKRVEKL (SEQ ID NO. 23)

WINZIPB11: VDELRAEVDQLEDENYALRTRVAQLRKQVEKL (SEQ ID NO. 24)

Trinucleotide codons (27) were used to code for randomized positions, all other positions were made with mononucleotides.

Library A:

TACTGTGGCGCAACTGNNNGAANNNGTGAAAACCCTTNNNGC-TNNNXXXTATGAACTTNNNTCTNNNGTGAGCGCTTGNNNGAGNNNGT TGCCCAGCTTGCTA (SEQ ID NO. 25) (encoding

VAQLXEXVKTLXAXZYLXSXV QRLXEXVAQL (SEQ ID NO. 26), wherein X represents a mixture of E, K, Q, and R, and wherein Z represents a mixture of N and V); libraryB:

CTCCGTTGACGAACTGNNNGCTNNNGTTGACCAGCTGNNNGACNNNX XXTACGCTCTGNNNACCNNNGTTCGCAGCTGNNNAAANNNGTGAAAA

AGCTGTGATA (SEQ ID NO. 27) (encoding
VDELXAXVDQLXDXZYALXTXVAQL- XKXVEKL (SEQ ID NO. 28),
wherein X represents a mixture of E, K, Q, and R, and wherein Z represents a
mixture of N and V) (NNN = equimolar mixture of the trinucleotides AAG, CAG,
GAG, CGT; XXX = equimolar mixture of the trinucleotides AAT, GTT).

Generation of the second strand and introduction of SalI and NheI restriction sites
were achieved by PCR using the primers prA-fwd:

GGAGTACTGGCATGCAGTCGACTACTGTGGCGCAACTG (SEQ ID NO. 29) and prA-rev: GGACTAGTACCTTCGCTAGCAAGCTGGGCAAC (SEQ ID NO. 30) or prB-fwd:

GGAGTACTGGCATGCAGTCGACCTCCGTTGACGAACTG (SEQ ID NO. 31) and prB-rev: GGACTAGTGCTAGCTTCTGACAGCTTTTCCAC (SEQ ID NO. 32), respectively. This resulted in a 142 bp double-stranded oligonucleotide for either library.

LibraryA and B were both digested with SalI and NheI, gel purified and ligated to the appropriate vector (Fig 2) yielding the plasmids LibA-DHFR[1], LibB-DHFR[2], LibB-DHFR[2:I114A] (Fig. 2A). After subcloning, the resulting linker between either library and DHFR fragment was: A(SGTS)₂STSSGI (SEQ ID NO. 33) for LibA and SEA(SGTS)₂STS (SEQ ID NO. 34) for LibB. To achieve maximal library representation, the ligation mixes were individually electroporated into XL1-Blue cells and selected with ampicillin on rich medium (LB). A 2- to 7-fold over-representation of each library was obtained. The resulting colonies were pooled and the plasmid DNA purified such that supercoiled plasmid DNA was obtained for cotransformation. The supercoiled DNA was cotransformed in BL21 cells yielding about 4×10^6 double-transformants. We used BL21 cells with a transformation efficiency of no less than 5×10^7 transformants per mg of DNA using 200 pg of DNA, or 2×10^7 transformants per mg using 500 ng of DNA. In cotransformations, the occurrence of double transformation was calculated as the number of colonies growing under selective pressure with trimethoprim (described below) divided by the number growing in the absence, when cotransformed with equal amounts of each DNA of a given, pre-selected pair. In order to verify that the library populations encode the designed amino acids with the expected frequency, single clones from each library were randomly picked and sequenced before selection. No statistically significant biases were detected. Seventy to 80% of each library had no mutations or frame-shifts, and thus the library-vs-library combination yielded approximately 50% correct sequence combinations. Thus, the experimental library-vs-library size of correct pairs is estimated as 2×10^6 .

The peptides WinZip-A1: Ac-STTVAQLEEKVKTLRAQNYELKSRVQRLRE-QVAQLAS-NH2 (SEQ ID NO. 35) and WinZip-B1: Ac-STSVDELQAEVDQLQDENYALKTKVAQLRKKVEKLSE-NH2 (SEQ ID NO. 36) were synthesized (Applied Biosystem 431A) and purified by reversed-phase HPLC. Electrospray mass spectrometry confirmed purity and identity of the peptides with a mass deviation of less than 1 Da. Peptide concentrations were determined by tyrosine absorbance in 6 M GdnHCl (39).